DRAFT FINAL REPORT

Bat Conservation International-University of Tennessee at Martin Wildlife Use of Livestock Troughs and Wildlife Escape Structure Study

7 December 2018

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THE UNIVERSITY OF TENNESSEE
MARTIN

Abstract

Many factors influence availability and quality of natural water sources that wildlife might use on the landscape. However, when natural water sources are unavailable or undesirable, wildlife may opportunistically exploit artificial water sources provided for livestock. In 2016, we collected survey data from 269 NRCS employees regarding the incidence of livestock producer reports of wildlife mortalities in livestock water troughs located east of the Mississippi River; 36.8% reported they or their producers observed dead animals in livestock troughs. In addition to the survey, Summer 2016, Summer 2017, and April 2018, we collected field data at livestock water troughs located in several states east of the Mississippi River. We examined the frequency of wildlife visits to troughs, the type of wildlife using these troughs, and the trough characteristics. During 48-hour sampling periods, we recorded wildlife use at each trough with trail cameras, and recorded bat activity and species richness in the vicinity of the trough with bat detectors. Several species of wildlife, the majority of which were either mammalian or avian, were observed using and/or interacting with more than two-thirds of the livestock water troughs in our study. The level of wildlife use of water troughs observed in this study suggests that livestock troughs might be an important alternative source of water for some wildlife species, even on landscapes where natural water sources are not limited.

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Introduction

Many factors influence availability and quality of natural water sources that wildlife might use on the landscape. However, when natural water sources are unavailable or undesirable, or even when they are available, wildlife may opportunistically exploit artificial water sources provided for livestock (Krausman et. al. 2006; Rosenstock et. al. 1999). As livestock troughs are not designed with wildlife use in mind, smaller wildlife are at risk of becoming immersed and drowning in the troughs while attempting to drink or bathe (Tuttle et. al. 2006).

Taylor and Tuttle (2007) published a set of guidelines for livestock producers in the Western U.S. regarding making livestock water developments safer and more accessible to wildlife. As a part of this *Water for Wildlife* publication (Figure 1), Taylor and Tuttle (2007) recommended methods for reducing obstructions to wildlife access, for maintaining water levels in livestock troughs, and for providing useful water sources for wildlife. In addition, this program promoted an effective, inexpensive wildlife escape structure to allow smaller wildlife trapped in steep-sided livestock troughs to climb out via the escape structure (Figure 2). These escape structures became mandatory for USDA Natural Resources Conservation Service (NRCS)-funded livestock troughs in the Western U.S., and the escape structures were recommended by the U.S. Forest Service (USFS), the Bureau of Land Management (BLM), and other agencies. However, no agreements were reached for wildlife escape structures for NRCS-funded livestock troughs located in the Eastern U.S.

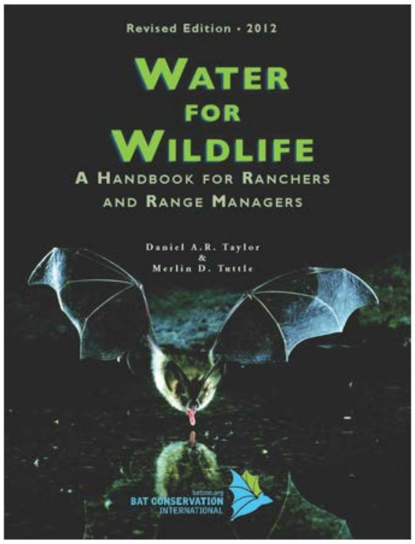


Figure 1. Bat Conservation International's *Water for Wildlife* publication (Taylor and Tuttle 2007).

Figure 2. Wildlife escape structure on livestock trough in the Western U.S. (photo courtesy of Dan Taylor, BCI).

Although natural water sources are more readily available on the landscape in the states east of the Mississippi River, wildlife might still opportunistically use livestock troughs as water sources due to their availability (Rosenstock et. al. 2004), their consistent supply of clean water, and/or a reduction of depredation risk while traveling on the landscape. This collaborative study examined the potential need for wildlife escape structures on livestock troughs in the states east of the Mississippi River. We examined the hypothesis that wildlife do use livestock troughs as a source of water, and predicted that medium to small-sized wildlife would use the troughs more frequently than larger-sized wildlife that could travel more safely to natural water sources on the landscape. Finally, we make recommendations regarding the need for wildlife escape structures on livestock troughs in states east of the Mississippi based on the results of this study.

Methods

Survey to NRCS employees

In April 2016, we developed a survey for distribution to NRCS employees regarding wildlife use and mortality at NRCS funded water developments (Appendix 1). This survey was reviewed and approved by the UTM Institutional Review Board (IRB) before being submitted to USDA NRCS for distribution (IRB#16-495-E054010). The survey was sent out by NRCS to NRCS employees in July 2016.

Collection of Field Data

During July – September 2016, June – September 2017, and April 2018, Nancy Buschhaus (PI) and Russell Milam (undergraduate student) of the University of Tennessee at Martin (UTM) collected data at 32 livestock water troughs owned by livestock producers located in four states (TN, KY, GA, and FL). We examined the frequency of wildlife encounters with a trough, the type of wildlife using these troughs, and the characteristics of each trough. We recorded wildlife use by recording activity with still photos and video for 48 hours at each water trough using three game cameras per trough, at varying distances and angles. A minimum of 288 minutes of video and photo samples were collected per 48-hour period per trough. We used a bat detector at each trough to record bat activity in the local area for 48 hours to demonstrate the presence/absence of bats as well as to record the species richness of bats found in the area. Finally, we collected water samples from each of the troughs for potential future eDNA analysis.

<u>Study sites and selection of sampling areas</u>
Unlike in the Western U.S., public lands with grazing leases are very uncommon in the Eastern U.S. Therefore, to sample active livestock troughs we had to gain access to private lands. To begin the study, Buschhaus and/or Milam contacted state- and local-level NRCS, Farm Bureau, and County Extension offices in TN, KY, GA, and FL to ask agency personnel to “get the word out” to their livestock producers regarding our study. In turn, due to privacy laws, we asked livestock producers to directly contact us via

phone and email, if they were interested in being a part of the study. Although all producers were offered anonymity, with no data being reported that would link the information to their particular location, upon further reflection, about half of those producers who contacted us or indicated interest in the study decided to decline participation.

Livestock producers' troughs sampled in Tennessee were more numerous than other states (N = 20) possibly due to more people being familiar with UTM in Tennessee. Troughs in TN were sampled summer 2016 and summer 2017 and were primarily located in counties west of the Tennessee River (Hardeman, Carroll, Henry, Montgomery, Obion, Weakley). Four troughs each in each of the remaining states (KY, N=4; GA, N=4; and FL, N=4) were sampled in summer 2016 (KY and GA) and April 2018 (FL).

<u>Trough and site characteristics</u>

The characteristics of each livestock trough were recorded at the beginning of each sampling period. These characteristics included type of trough (open-water or ball-type automatic waterer), material of trough (polyethylene, galvanized steel, fiberglass, other plastics, cement), shape of the trough (round, rectangular, oval), size of the trough (in gallons), height of the trough (in cm), length of the trough (in cm), distance from rim to water level at beginning of sampling period (in cm), presence or absence of structures immediately over or around edge of trough, distance to nearest natural water source (in meters), distance to nearest treeline (in meters), distance to nearest building (in meters), and distance to nearest active residence (in meters).

<u>Wildlife Cameras (scan samples, videos of behavior, IR sampling)</u>

Three Wildlife cameras (Bushnell Wildlife Trophy Cam™ with field scan mode) were used to examined the frequency of wildlife encounters with a trough and the type of wildlife using these troughs. We recorded wildlife use by recording activity with still photos and video for 48 hours at each water trough using three game cameras per trough, at varying distances and angles (Figure 3).

Figure 3. Position of wildlife cameras (circled in yellow) and bat detector (circled in blue) in relation to the trough.

Of the three cameras, Camera One was placed within 2 m of the trough and was set on field scan mode to take one picture once every 60 seconds for 48 hours. Camera Two was placed directly opposite the first camera, within 2m of the trough, and was set on field scan mode to take one 30 second video every 5 minutes. Camera Three was placed 20 m away from the trough, to the right or the left of the front of the trough (so that it could see all of the trough as well as the cameras and bat detector near the trough), and was set on regular IR (infrared) mode to be triggered and take pictures only when an endotherm entered the field of view.

Due to the nature of the IR sensor, we took both scan samples (Camera One) and focal samples (Camera Two) of the trough. Wildlife cameras are a relatively inexpensive way to take pictures of wildlife because they have both the video and picture capability to film in low light with IR lighting. However, the IR sensor is limited in warm environments because the triggering capability requires that there is enough of a temperature differential between the endotherm in view and the background temperature. Therefore, on warm days and nights, smaller endotherms like birds and bats generally will not trigger the IR sensor. So, the scan samples (one picture every minute for 48 hours) and the focal samples (one 30 second video every 5 minutes for 48 hours) take advantage of the IR lights and camera without having to purchase more expensive camera and IR lighting.

Whenever possible, pictures and video of wildlife were used to identify species.

<u>Bat Acoustics (survey and analysis)</u>

We used a Wildlife Acoustics SM4 full spectrum bat detector with an omnidirectional microphone that generally records bat passes within 30m of the microphone. We placed the detector and microphone within 2m of each trough to record bat activity in the local area for 48 hours to demonstrate the presence/absence of bats as well as to record the species richness of bats found in the area. We used Sonobat 4.2.1 North America version with appropriate regional classifiers to visualize bat passes, automatically identify calls, and count the number of bat passes for bat activity. Buschhaus manually vetted the calls that were of high enough quality to determine species presence.

<u>Water samples for eDNA</u>

At the end of each 48-hour sampling period, we took a water sample from the trough for eDNA sampling. The water sample was collected by first reaching into the trough and stirring the water in the trough, then we placed an autoclaved 1L Nalgene sampling bottle that had been rinsed with 70% ethanol (and dried) down into the water until at least ¾ full. We capped bottle, brought it back to the laboratory, and froze the water sample at -80°C.

We submitted the samples to the Northern Arizona University Bat Ecology and Genetics Laboratory (PIs Drs. F. Walker and C. Chambers) for Nex Gen sequencing and determination of species using the water troughs. To prepare these samples for shipping, we first flash-thawed the frozen water samples in a 60°C (to denature any enzymes that would break down DNA that were released when cells in the water lysed upon freezing). We then, using sterile techniques including rinsing instruments in ethanol then flaming between samples, filtered water samples across acetate filters with pore sizes small enough to capture any DNA and/or cellular debris. Each filter dried in the process of the extraction preserving the DNA on the filter for future use. Filters were then packaged individually in foil wrap and sent to the NAU laboratory for future analysis.

At NAU, the samples were PCR amplified with 12s mammal primers (https://www.mdpi.com/1424-2818/9/4/54). Resulting amplicons were subjected to Illumina MiSeq next generation amplicon sequencing. The sequences were quality filtered and dereplicated using Mothur scripts, and taxonomies were assigned using the NCBI BLAST remote suite. One non-template control had slight amplification and was sequenced to ensure no contamination was present in other samples. This was confirmed during the final analysis.

Subsample of repeated troughs in year 2

Seven troughs that were sampled in 2016 were again sampled in 2017 using the same methods as mentioned above to determine whether there were any patterns in wildlife use between years.

Results

Survey to NRCS employees

Due to communication complications with NRCS, the survey response rate and survey period were less than originally planned. However, we still received 275 responses from 24 states from 764 employees who received the survey. Perhaps most importantly, 99 respondents (36.8%) said they or their producers had found dead wildlife in their troughs at some point (Figure 4).

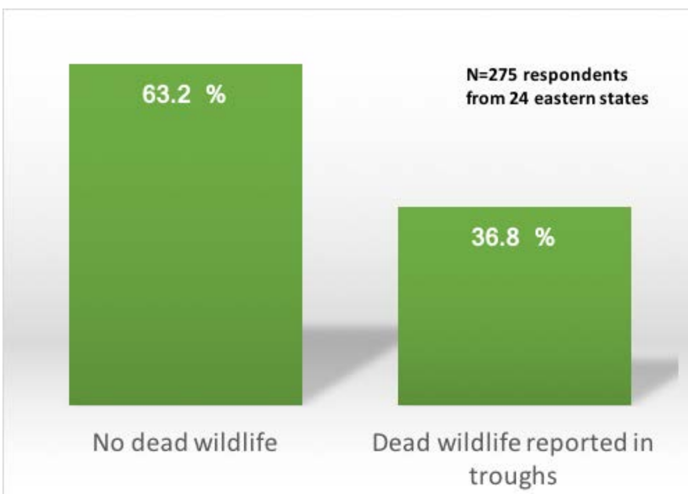


Figure 4. The percentage of NRCS employee responses regarding whether dead wildlife had been observed by the employee or livestock producer in the producer's livestock troughs.

Field Data

Trough and site characteristics

The majority of troughs sampled in the study were 75-100 gallon troughs. Most were open-water, oval (31”x53”x25”) or round (63”d x 25”) shaped polyethylene (primarily constructed by Rubbermaid™) (N=20), but a few were open-water, oval or round, galvanized metal (N=6), round cement (N=4), or polyethylene, ball-type, automatic waterers (N=6). Both open-water troughs and ball-type automatic waters had some incidence of wildlife on or in the trough (Figure 5).

Figure 5. Mammalian and avian wildlife using open water and ball-type automatic waterer as a water source.

The majority of troughs had some structure (mostly fencing) over the top of the trough (so that livestock on both sides of the fence had access to the water; see Figure 3) or against one side of the trough (when the trough was placed against the fence).

All of the livestock troughs were within 500m of an active natural or man-made water source, and just under half were located within 100m of a non-residence building (e.g. barn or shed). Very few troughs were located within 100m of an active residence.

Wildlife Cameras (scan samples, videos of behavior, IR sampling)

The total wildlife observations per trough were taken from 2880 scan sample pictures (one picture per minute for 48 hours), 576 thirty-second focal sample videos (30 seconds of video every 5 minutes for 48 hours), and multiple IR-triggered videos and pictures (triggered by wildlife such as raccoons that were large enough to set off the IR motion sensor).

Of the 32 troughs, 26 had photo or video evidence of wildlife either drinking from or sitting/walking on the trough (Figure 6).

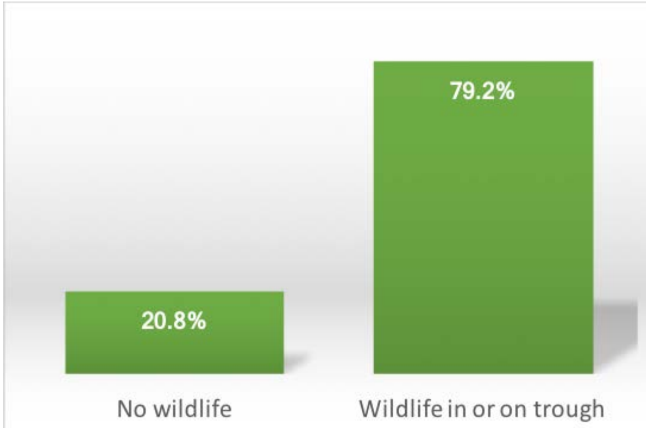


Figure 6. The percentage of troughs with and without photo/video evidence of wildlife in and/or on the troughs.

Birds were the most common endotherm using and/or sitting on the troughs (Table 1).

Table 1. Bird and mammal species (endotherms) using or interacting with the troughs.

Species Name	**Common Name**
Corvus brachyrhynchos	American Crow
Cardinalis cardinalis	Northern Cardinal
Molothrus ater	Brown-headed Cowbird*
Mimus polyglottos	Northern Mockingbird
Zenaida macroura	Mourning Dove
Procyon lotor	Raccoon*
Sciurus niger	Fox Squirrel
Sciurus carolinensis	Gray Squirrel
Didelphis virginiana	Virginia Opossum
Marmota monax	Groundhog

*most common mammal and most common bird observed at the troughs

Of the observations, 297 pictures and/or videos observed endotherms on a total of 64 sampling days (each sampling day included both day and night observations for 24 hours for two days total) for an average of 4.64 observations of endotherms per sampling day. Of those, birds were much more common than mammals, with 259 observations of the total 297 of endotherms (87.2%) being birds of various species (Figure 7). Mammal observations, made up 38 of the 297 observations (12.3%), and by far, most of those observations were of raccoons, *Procyon lotor*. Almost all of the 26 troughs had both bird and raccoon sightings; some also had additional mammal sightings at the same trough.

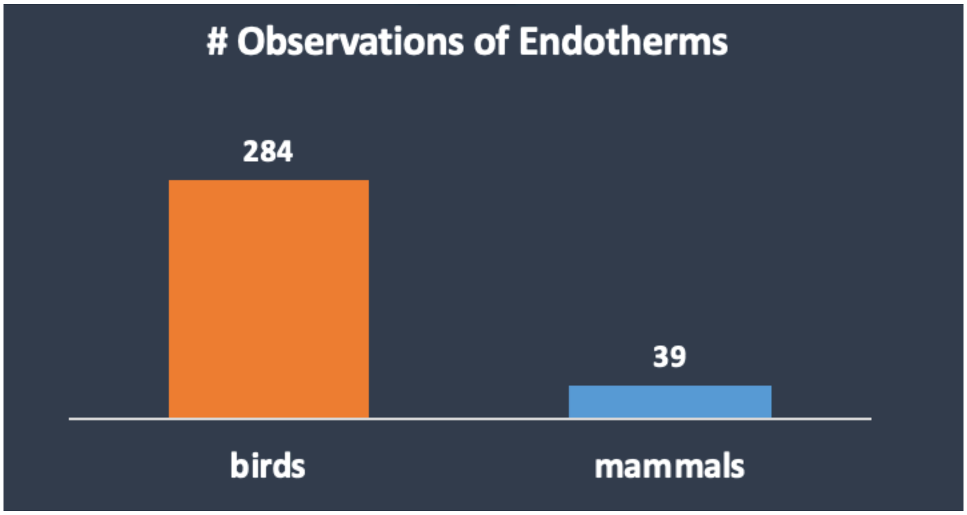


Figure 7. Observations of endotherms visiting the troughs during the study.

Two of the 32 troughs had significant ectotherm observations. Since we were not relying on the IR sensor, we could observe these organisms in addition to those of endotherms. In fact, 893 total observations of mostly frogs and two salamanders from 4 sampling days (223.3 average observations per sampling day; Figure 8) dominate the total number of observations of all organisms (1291 observations overall) primarily because the frogs and salamanders were present for multiple hours of sampling time. Based on the videos captured at these two troughs the frogs and salamanders were primarily engaged in mating behaviors associated with these two troughs. Finally, there was a single observation of an Eastern Fence Lizard, *Sceloporus undulatus*, at a different trough, as well.

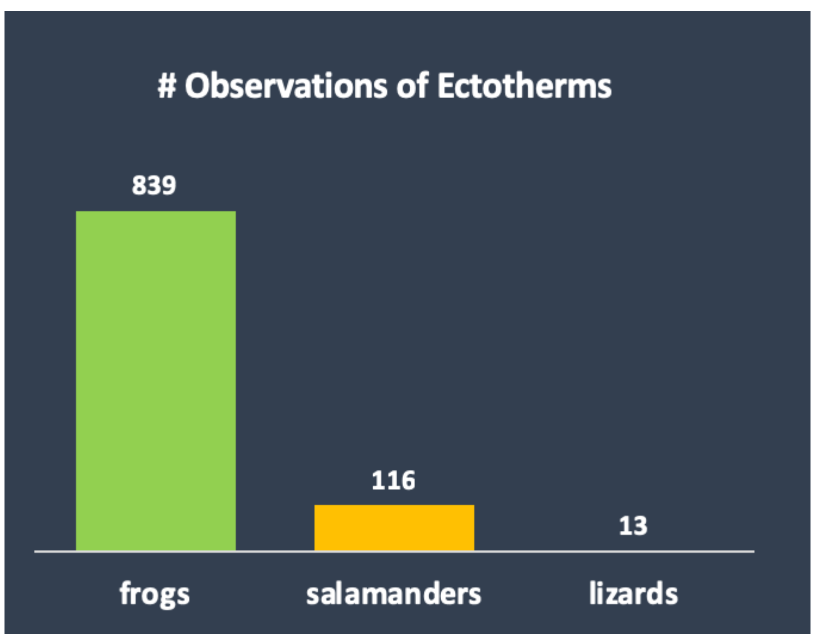


Figure 8. Observations of ectotherms visiting the troughs during the study.

Bat Acoustics (survey and analysis)
No species of bats were recorded using the livestock trough. However, many troughs had relatively high levels of bat activity in the vicinity. In general, each night of sampling during the summer sampling periods yielded about 100-4100 bat passes per night. Those recorded bat passes were analyzed by SonoBat and manually vetted by Buschhaus. Bat passes of high enough quality yielded the presence of at least 8 species of bats that were foraging within 30m of the detector microphone (Table 2).

Table 2. Bat species recorded during the study.

Species Name	**Common Name**
Lasiurus borealis	Eastern red bat
Nycticeius humeralis	Evening bat
Perimyotis subflavus	Tri-colored bat
Lasionycteris noctivigans	Silver-haired bat
MyLu/MySo	Little brown/ Indiana bat
Myotis austroriparius	Southeastern myotis
Lasiurus cinereus	Hoary bat
Eptesicus fuscus	Big Brown bat

Repeat sampling for subsample of troughs in year 2
Russell Milam (undergraduate student; now a graduate student at Murray State University) used the data set associated with a small number of troughs (N=7; Figure 9) that were resampled in year 2 (Summer 2017) to analyze the factor that was most associated with repeat visits both between nights and between years (generalized linear model with Poisson distribution).

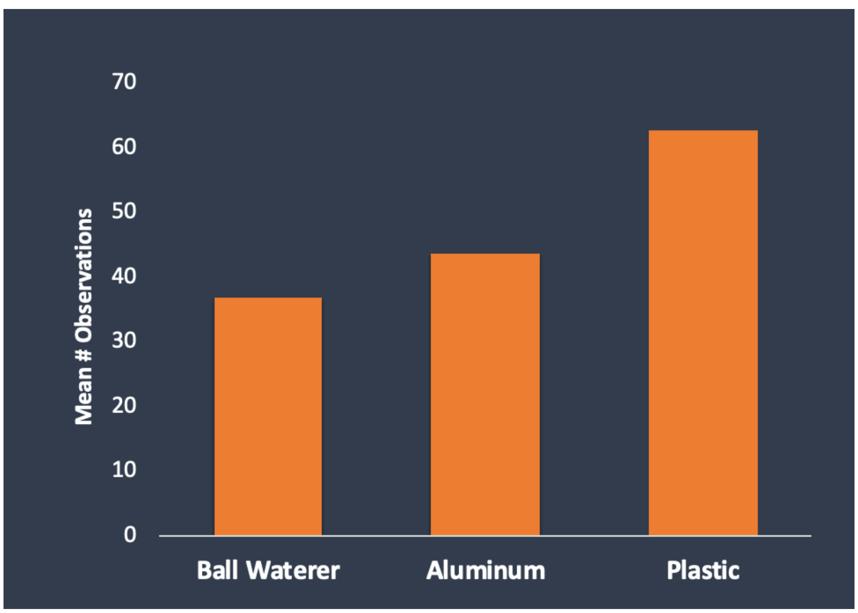
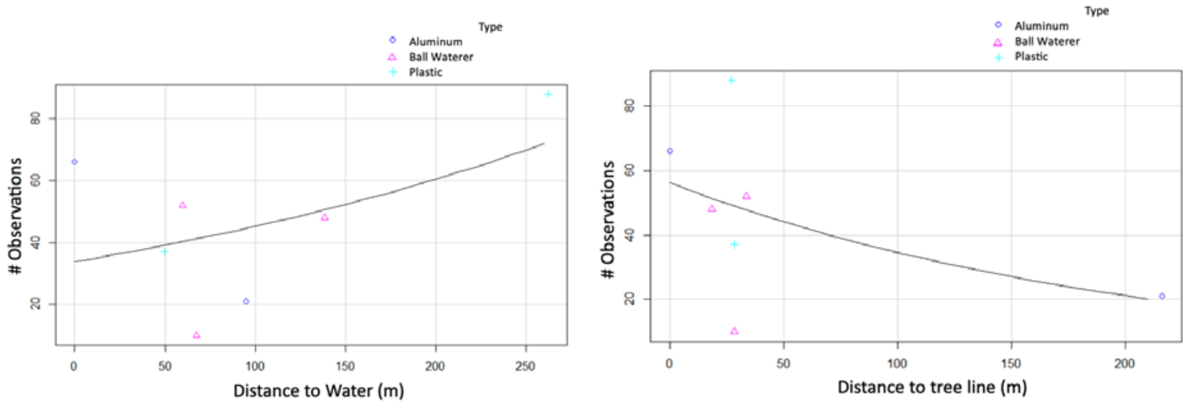


Figure 9. Mean number of repeat wildlife observations at resampled troughs (N=7) by trough type.

While the overall likelihood of a trough's use by wildlife was not related to the distance to the nearest water source nor the previous precipitation pattern, Russell found that livestock troughs that were farthest from natural water sources were most likely to have multiple visits between nights and between years (Figure 10; r2=0.23; p<0.001) and those that were farthest from the natural tree line had fewer wildlife visits between nights and between years (Figure 11; r2=0.20, p<0.001). While it is difficult to assess whether unmarked animals were using the troughs more than once in the 48-hour sampling period, the same species of birds tended to use the troughs near the same time each day at several of the troughs.



Figures 10 and 11. The effect of distance to water (m) and distance to tree line (m) on the number of wildlife observations at water troughs.

<u>Environmental DNA (eDNA) analysis</u>
The Northern Arizona University Bat Ecology and Genetics lab reported the following regarding the eDNA analysis.

Bat taxa were not identified in the samples. However, we (NAU) detected the following taxa, ordered here from the most to least abundant. Human and bacterial sequences dominated. In parentheses next to each item are interesting genera or families.
Human
Bacterial genera
Dog (*Canis*)
Cow (*Bos*)
Ungulate
Rodent (*Mus*)
Avian (*Meleagris*, *Podicipediformes*)
Fish (*Amia*)
Algae and water plants
Unidentified chordate environmental sequences and unidentified bacterial sequences

Products of the study

This project has resulted in several presentations.

Milam, R., D. Taylor, S. Tuttle, M. Miller, and N. Buschhaus. 2017. Wildlife use of livestock water troughs in several states east of the Mississippi River. Poster. Kentucky Bat Working Group Meeting. November 2018.

Buschhaus, N., R. Milam, D. Taylor, and S. Tuttle. 2018. Wildlife use of livestock water troughs in several states east of the Mississippi River. Poster. American Society of Mammalogists National Meeting. Kansas State University, KS. June 2018.

Milam, R., D. Taylor, S. Tuttle, M. Miller, and N. Buschhaus. 2017. Wildlife use of livestock water troughs in several states east of the Mississippi River. Poster. North American Society for Bat Research. Knoxville, TN. October 2017.

Taylor, D., N. Buschhaus, S. Tuttle, and R. Milam. 2017. Wildlife use of livestock water troughs in the southeastern U.S. Paper. National Wildlife Society Meeting, Albuquerque, NM. September 2017.

Milam, R., D. Taylor, S. Tuttle, M. Miller, and N. Buschhaus. 2016. Wildlife use of livestock water troughs. Poster. Tennessee Academy of Sciences and TN Bat Working Group Meetings. November 2016.

Discussion

Survey to NRCS employees

Based on the survey results, it is clear that at least in some years, wildlife mortality does occur in livestock water troughs, most likely among smaller wildlife species (personal communications: several of the livestock producers who participated in this study mentioned that during drought years it was not uncommon to occasionally find squirrels, chipmunks, and/or birds dead in their troughs) that would have difficulty escaping the trough should they fall in during a visit to the trough.

Field study of Wildlife Use of Water Troughs

Based on the field study, it is clear that even in a landscape with nearby natural and manmade water sources, medium to small-sized wildlife do still use livestock water troughs.

The distance to the nearest water source nor the previous precipitation pattern were not related to the likelihood of wildlife use, although for those troughs where we sampled both years, distance to nearest water source had an inverse relationship with likelihood of repeat visits. There were trends that indicated that the type of trough might be related to likelihood of wildlife use (Rubbermaid/plastic/poly blend more likely than cement), as was the distance to nearest human habitation (the closer to human habitation, the less likely to have wildlife interacting with the trough), but additional data is necessary before we can determine if this relationship is statistically significant. However, no single factor was related to likelihood of non-use by wildlife.

The wildlife use of water troughs observed in this study suggests that livestock troughs might be an important alternative source of water for some wildlife, especially moderate- to small-sized wildlife (Krausman et. al. 2006) , for whom predation risk might increase as they have to cross the landscape to reach the nearest natural water source. In fact, in the limited sampling of troughs between years, the longer the distance to the nearest treeline, the more likely a trough was to have multiple visits between nights and between years.

Therefore, these opportunistic interactions by wildlife with the artificial water sources may also increase the risk of mortality in livestock water troughs (Rosenstock et. al. 2004; Tuttle et. al. 2006) that do not have wildlife escape structures. Additional sampling from the field and the survey results may elucidate the likelihood of risk of wildlife mortality in livestock water tanks and the need for wildlife escape structures in livestock water troughs in the eastern United States.

Recommendations

All four states had at least some troughs that received visits by wildlife; and in the TN group of troughs, all but 2 of the 20 troughs had visits by wildlife. Based on the frequency of troughs that had visits by wildlife, I think that it is clear that requiring wildlife escape structures on NRCS-funded troughs would be good practice, especially for states found in the southeastern U.S.

Acknowledgements

Charles Rewa, USDA-Natural Resources Conservation Service

Stuart Tuttle, USDA-NRCS

Marcus Miller, USDA-NRCS

GA, KY, and TN USDA-NRCS Field Officers that helped ”get the word out” about this study to livestock producers

UTM College of Engineering and Natural Sciences Blankenship Undergraduate Research Award

The private landowners who allowed us access to their land

Appendix 1 Survey Preamble and Questions

Dear NRCS Colleagues,

Bat Conservation International (BCI) and the University of Tennessee-Martin (UTM) are receiving support from the NRCS CEAP Program to help determine if wildlife escape structures (WES) are necessary in NRCS-funded livestock water troughs east of the Mississippi River.

From 2004-2005, research and field investigation conducted in 11 western states by the NRCS, BCI, and several agency partners determined that more than 10% of all steep-sided livestock and wildlife watering developments were trapping and drowning bats, birds, small mammals and other wildlife that accidentally fell in the water while attempting to drink or bathe, including rare and protected species. With hundreds of thousands of troughs on the landscape, this can be considered a significant loss.

As a result, the NRCS developed Conservation Practice Standards requiring the installation of WES's in all NRCS-funded steep-sided livestock and wildlife water developments in these states. Several NRCS Technical Center, State, and Field Office staff have recommended that this Conservation Practice Standard be applied nationwide, while others have suggested that wildlife mortality in water developments in the east is negligible, making the use WES's unnecessary.

The following survey developed by BCI, UTM, and NRCS Technical staff is the first step in an evidenced-based study designed to collect information upon which to make a well-informed decision. Please complete and submit the survey by **Wednesday, May 25**. Participation in the survey is voluntary. If you decide to participate, you may withdraw from the study at anytime without penalty and without loss of benefits to which you are otherwise entitled. Participant information will be kept confidential, and only the survey responses will be sent to the researchers.

These results will help us to plan the second phase of the study; field visits to willing landowners in the east of the Mississippi River to collect site-specific information. Your assistance in helping to identify landowners who might be willing to allow PI/student researchers to conduct further wildlife trough investigations on their property would be greatly appreciated. Thank you for your assistance.

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Wildlife use of Troughs

2 / 4 50%

*** 1. In which state are your producers located in?**

2. What counties are your producer's located in?

County 1

County 2

County 3

County 4

County 5

Prev Next

USDA

Wildlife use of Troughs

3 / 4 75%

* 3. How many of your producers have, or do you estimate have, water troughs (i.e. steep-sided water receptacles, not ponds)?

* 4. What percent of all of your producers have, or do you estimate have, water troughs (i.e. steep-sided water receptacles, not ponds)?

* 5. What types of water troughs are most commonly used by your producers?

- [ ] Metal, round/oval
- [ ] Metal, rectangular
- [ ] Cement, round
- [ ] Cement, rectangular
- [ ] Fiberglass, rectangular
- [ ] Plastic/poly
- [ ] Other (please specify)

* 6. What is the average size of the area of water surface in the water troughs used by

your producers (length x width in square feet for rectangular, oval or square tanks; diameter in feet for round tanks)?

7. What percentage of producers in your area use wildlife escape structures (i.e. ramps, bird ladders)?

8. Have any of your producers told you that they have found dead animals in their troughs, or have you ever observed dead animals in their troughs?

- ◯ Yes
- ◯ No

Comments if any:

9. What percentage of the tanks are prone to fouling due to:

Algal Growth

Overhead Vegetation

Dead Wildlife

Other (Enter the source and the percentage)

Prev Done

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